REMARKS

Claims 14-94 having been canceled, claims 1-13 are pending.

Examiner Interview Summary

Examiner Pak and Supervisory Patent Examiner Achutamurthy are thanked for the courtesy of the telephone interview with Applicants' Representative Victoria Sandberg and inventor Dr. Friedrich Srienc on April 6, 2006. All claims were discussed. The parties to the interview discussed the impact of Madison et al. and Linde et al. on patentability, particularly as they relate to *anaerobic* production of PHA in yeast. The Examiners suggested that Applicants present their further arguments in written form as a response to the outstanding office action.

Rejection under 35 U.S.C. §103

The rejection of claims 1-13 under 35 U.S.C. §103(a) as being unpatentable over Madison et al., Johnston et al., Clemente et al. and Linde et al. was maintained by the Examiner. This rejection is respectfully traversed. The Examiner is invited to review Applicants' arguments presented in the Response filed June 24, 2006, which are incorporated herein by reference and supplemented below.

In order to establish a *prima facie* case of obviousness, three criteria must be met. First, there must be motivation to combine the references relied upon. Second, there must be a reasonable expectation of success in making the claimed invention. And third, the combination of references must teach all of the elements of the claimed invention. MPEP §2143. Applicants respectfully submit that the Examiner has not met this burden.

Claim 1, from which the other pending claims depend, reads as follows:

> 1. (Original) A method for the production of a polyhydroxyalkanoate (PHA) comprising:
>
> providing a transgenic yeast cell comprising a first nucleic acid fragment comprising a heterologous nucleotide sequence encoding a PHA polymerase and

at least one second nucleic acid fragment comprising a heterologous nucleotide sequence selected from the group consisting of a heterologous nucleotide sequence encoding an acetoacetyl-CoA reductase and a heterologous nucleotide sequence encoding a β-ketothiolase;

culturing the transgenic yeast cell under anaerobic conditions to cause the producn of PHA; and

isolating the PHA from the yeast cell.

Applicants submit that the cited art, either alone or in combination, does not teach or suggest culturing a transgenic yeast cell under *anaerobic conditions* to cause the *production of polyhydroxyalkanoate*.

The primary reference, Madison et al., teaches production of a PHA in transformed *S. cerevisiae*. However, as acknowledged by the Examiner, Madison et al. does not teach *anaerobic* production of PHA in yeast. To provide this missing element, the Examiner cites Linde et al., stating that "Linde teaches flexibility of expressing genes in anaerobic and aerobic culture conditions" (Office Action mailed August 2, 2005).

Applicants respectfully submit, however, that the teachings of Linde et al. fall far short of remedying the deficiency of Madison et al. Anaerobic cultures of yeast are, without dispute, in the prior art. What is at issue here, however, and what is claimed, is the *production of polyhydroxyalkanoate* under anaerobic conditions in transgenic yeast. Successful production of a polyhydroxyalkanoate in yeast under *aerobic* culture conditions (Madison et al.) would no more provide a reasonable expectation of successful production of PHA under anaerobic conditions, than successful production of ethanol under anaerobic conditions would provide a reasonable expectation of success that ethanol could be produced under aerobic conditions. This is because polyhydroxyalkanoate is not just a metabolite whose production might be incidentally, if at all, affected by the energy pathway utilized by the cell; rather, it is, like ethanol, a compound that Applicants show is *directly involved* (e.g., as an electron sink or fermentation product; see specification at page 11, lines 21-25) in a metabolic pathway involved in energy production and utilization.

PHA's direct involvement in the process of energy production for a yeast cell makes it unreasonable to extrapolate from production of PHA in aerobic culture to production in anaerobic culture, as the Examiner has done in combining Madison et al. and Linde et al. The significance of PHA as an energy-related metabolite is emphasized in several places in the specification, for example:

> Anaerobic production of PHA in microorganisms is *surprising* because PHA is typically considered an *aerobic storage material* in microorganism cells. Moreover, β-oxidation of fatty acids is an aerobic process, and removing oxygen from the system might have been expected to reduce the precursor pool and thereby *inhibit* PHA production (specification at page 15, lines 7-11; emphasis added).

Thus, it was surprisingly found that:

> *[d]uring anaerobic growth, PHA can act as an electron sink or fermentation product*, and it is possible to couple PHA production with the production of one or more additional electron acceptor compounds ("electron sinks" or "redox sinks") such as ethanol or lactic acid (specification at page 11, lines 21-25).

And:

> Biological co-production of products in addition to PHA is also encompassed by the invention. Preferably, these co-products are produced during anaerobic fermentation and serve as electron receptors or "electron sinks". For example, microorganisms can co-produce ethanol or lactic acid along with PHA (FIG. 4). *Under aerobic conditions, the accumulation of PHB typically correlates with a reduction in ethanol concentration. However, we discovered that under anaerobic conditions, ethanol production is linked to PHA accumulation* (specification at page 15, lines 23-30; emphasis added).

And further, with respect to the delicate redox balance and energy metabolism in yeast:

> *Redox balance in yeast*
>
> Maintaining a favorable redox balance plays a critical role in an organism's metabolism. The over expression of a metabolite or the expression of a heterologous protein can have a significant effect on a host's redox balance.
>
> NADH and NADPH are the two major nicotinic cofactors used to shuttle reducing equivalents between enzymes. NADH is produced primarily from the

catabolism of energy sources and from the biosynthesis of some amino acids. During oxidative growth, the reducing equivalents carried by NADH can be transferred to the electron transport chain where O_2 is reduced to H_2O in a process that generates ATP. Anaerobic conditions pose a redox challenge because the electron transport system is not available to accept electrons. Instead, the reducing equivalents are transferred to a metabolite that acts as an electron acceptor (i.e., an "electron sink"). The reduced metabolites are then typically exported from the cell. The reduction of acetaldehyde to ethanol is a common example.

NADPH is the second major nicotinic coenzyme involved in redox balances. This coenzyme is used in anabolic reactions and in *S. cerevisiae*, is produced primarily by the hexose monophosphate pathway. Other enzymes like cytosolic isocitrate dehydrogenase and cytosolic acetaldehyde dehydrogenase also produce NADPH.

Due to the absence of a transhydrogenase system ($NADH + NADP^+ \leftrightarrow NAD^+ + NADPH$), NADPH and NADH are not equivalent in *S. cerevisiae*. The two cofactors must be synthesized and consumed in separate reactions.

Transhydrogenase systems

Perturbations in the concentrations of the NAD(P)H/NAD(P)+, such as those caused by the introduction of a catabolic or anabolic pathway can often lead to an unfavorable redox balance. In *S. cerevisiae*, the cell must regulate a delicate balance between the production and consumption of NADPH and NADH in order to maintain a favorable redox balance. Many bacterial and animal cells possess a transhydrogenase activity that permits the conversion of NADH to NADPH or *vice versa*, however *S. cerevisiae* does not possess this activity. The lack of a transhydrogenase system has implications on the expression of foreign proteins and on the yields of such products as ethanol. For instance, glycerol is produced during anaerobic growth in order to reoxidize the NADH formed during biomass production. Glycerol formation can significantly affect the economics of processes like ethanol production by lowering yields. (specification at page 20, line 23, bridging to page 21, line 27).

Further, although Linde et al. teach that "*[i]n quantitative terms*, the aerobic and anaerobic transcript profiles of *S. cerevisiae* exhibit little difference" (Linde at col. 2, p. 7412; emphasis added), they also note that a small number of genes exhibited a *greater than 10-fold difference* between aerobic and anaerobic mRNA levels. Among those included genes could be *directly linked* to typical aerobic process, among them genes involved in *β-oxidation*, including PXA1, a transporter involved in translocation of long-chain fatty acids across the peroxisomal

membrane, and FOX2, encoding 3-hydroxyacyl coenzyme A epimerase (Linde at col. 2, p. 7412).

As PHA is also an energy-related metabolite, Applicant contends that Linde does not support an expectation or conclusion of "little difference" in the production of PHA in anaerobic vs. aerobic conditions. Indeed, because it is a process affected by β-oxidation, an aerobic process, PHA production would, according to Linde et al., be *more likely* to be affected by change from aerobic to anaerobic culture conditions (see col. 2, p. 7411). Linde et al. thus actually *teach away from* the present invention by implying that an aerobic process such as fatty acid metabolism (and hence, PHA production) would, if anything, be adversely affected by anaerobic culture conditions.

Not only do Linde et al. fail to support a *reasonable expectation of success*, but further, in view of Linde's teaching of a drop in transcript levels associated with typical aerobic processes of over 10X, a skilled artisan *would not be motivated* by Linde et al. to combine the references to carry out an aerobic process such as PHA production under anaerobic conditions.

For at least these reasons, it is respectfully submitted that the cited art provides neither a motivation to combine the cited references, nor a reasonable expectation of success in achieving anaerobic production of PHA in yeast, and that the Examiner has failed to establish a *prima facie* case of nonobviousness. Reconsideration and withdrawal of the rejection of claims 1-13 under 35 U.S.C. §103 is, accordingly, kindly requested.

Conclusion

It is respectfully submitted that the pending claims 1-13 are in condition for allowance and notification to that effect is respectfully requested. The Examiner is invited to contact Applicants' Representatives at the telephone number listed below if it is believed that prosecution of this application may be assisted thereby.

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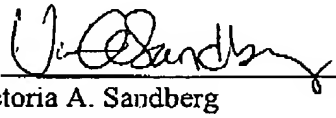
By: 
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CERTIFICATE UNDER 37 CFR §1.8:
The undersigned hereby certifies that the Transmittal Letter and the paper(s), as described hereinabove, are being transmitted by facsimile in accordance with 37 CFR §1.6(d) to the Patent and Trademark Office, addressed to Commissioner for Patents, Mail Stop AF, P.O. Box 1450, Alexandria, VA 22313-1450, on this 12th day of May, 2006, at 3:05 pm (Central Time).

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